Data Evaluation Record on the Acute Toxicity of BAS 781 02 H (formulation containing 54.6% Dimethenamid-P and 6.2% Saflufenacil) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0432
PMRA Document ID: 1662897 EPA MRID Number: 47560403

Data Requirement:	PMRA DATA CODE	9.8.6
	EPA DP Barcode	349851
	OECD Data Point	IIIA 10.2.2.3
	EPA MRID	47560403
	EPA Guideline	OPPTS 850.5400 (123-2)

Test material: BAS 781 02 H (AI: Saflufenacil) **Purity:** 54.6% (BAS 656 H; Dimethenamid-P) and 6.2% (BAS 800H)
Common name: Dimethenamid-P formulation
Chemical name: IUPAC: BAS 656 H: (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide; not reported for a.i. Saflufenacil
CAS name: Not Reported
CAS No.: BAS 656 H: 163515-14-8; not reported for a.i. Saflufenacil
Synonyms: None Provided

Moncie V Wright

Primary Reviewer: Moncie Wright **Signature:**
Staff Scientist, Cambridge Environmental **Date:** 11/11/08

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental **Date:** 11/17/08

Primary Reviewer: Anita Pease **Date:** 06/09/09 APease 6/9/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Ann Lee **Date:** 06/09/09
HC-PMRA-EAD

Secondary Reviewer: Farzad Jahromi **Date:** 06/09/09
DEWHA-APVMA

Company Code BAZ
Active Code SFF
Use Site Category: 13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code 118203

CITATION: Minderhout, T, Kendall, T.Z., Krueger, H.O., and C. Holmes. 2008. BAS 781 02 H: A 96-Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland, and sponsored by BASF Corporation, Research Triangle Park, North Carolina. Laboratory Project ID: Wildlife International Study No.: 147A-240A. BASF Study No.: 355544. Study completed August 28, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular

plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the freshwater algae (*Pseudokirchneriella subcapitata*) were exposed to BAS 781 02 H (formulation containing 54.6% Dimethenamid-p and 6.2% Saflufenacil) at nominal concentrations of 0 (negative control), 3.9, 6.5, 11, 18, and 30 µg form/L under static conditions. Only concentrations of dimethenamid-p were analytically verified. Given the stability of saflufenacil and dimethenamid-p in freshwater studies and lack of analytical verification for the formulation, all endpoints are reported in terms of nominal concentration.

Inhibitions of cell density ranged from 2.0 to 96%, inhibitions of biomass ranged from 5.8 to 96%, inhibitions of growth rate ranged from 0.33 to 55%, and inhibitions of yield ranged from 2.0 to 96%.

There were no signs of flocculation or aggregation of cells and no adherence to the test vessels in the controls and treatment groups. Cell enlargement was observed in the highest treatment level.

The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of 3.9 and 14 µg BAS form/L, respectively.

It can be concluded that both dimethenamid-p and saflufenacil contribute to the toxicity of BAS 781 based on comparison of results to the range of green algae 5 d EC_{50} values for technical dimethenamid-p (0.014 to 0.017mg a.i./L (e-Pesticide Manual; MRID 44332253) and the 96h EC_{50} for technical saflufenacil of 0.0462 mg a.i./L (IIA 8.4; MRID 471279-23; PMRA 1547225).

This toxicity study is classified as **ACCEPTABLE** to the **U.S EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for a nonvascular aquatic plant toxicity study with the freshwater algae, *Pseudokirchneriella subcapitata.*

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

BAS 781 02 H
Cell density

EC_{05}:	8.3 µg form/L	95% C.I.: 7.6 to 9.2 µg form/L
EC_{50}:	16 µg form/L	95% C.I.: 15 to 16 µg form/L

NOAEC: 3.9 µg form/L
Probit Slope: 6.05 ± 0.293

Biomass (Area Under the Growth Curve)

EC_{05}:	7.1 µg form/L	95% C.I.:	6.8 to 8.5 µg form/L
EC_{50}:	14 µg form/L	95% C.I.:	14 to 15 µg form/L

NOAEC: 3.9 µg form/L
Probit Slope: 5.40 ± 0.279

Growth Rate

EC_{05}:	13 µg form/L	95% C.I.:	11 to 15 µg form/L
EC_{50}:	28 µg form/L	95% C.I.:	28 to 30 µg form/L

NOAEC: 11 µg form/L
Probit Slope: 4.80 ± 0.331

Yield

EC_{05}:	8.4 µg form/L	95% C.I.:	7.6 to 9.3 µg form/L
EC_{50}:	16 µg form/L	95% C.I.:	15 to 16 µg form/L

NOAEC: 3.9 µg form/L
Probit Slope: 6.13 ± 0.303

Endpoint(s) Effected: Cell density, biomass (most sensitive), growth rate, and yield

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the OECD Guidelines for Testing of Chemicals, 201: "*Freshwater Alga and Cyanobacteria, Growth Inhibition Test*", Official Journal of the European Communities, No. L383, Method C.3: *Algal Inhibition Test*, US EPA Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400, *Algal Toxicity, Tiers I and II*, and ASTM Standard 1218-90E: *Standard Guide for Conducting Static 96-Hour Toxicity Test with Microalgae*. The following deviations from OPPTS 850.5400 were noted:

1. Pretest health of the test species was not reported.
2. The pH of the test concentrations was higher than the recommended value of 7.5 by study termination, with values ranging from 7.9 to 9.1.

These deviations do not affect the study acceptability.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, Certification, and Quality Assurance statements were provided. This study was conducted in compliance with U.S. EPA FIFRA GLP standards (40 CFR Part 160 and 792), OECD Principles of GLP and JMAFF GLP (1999), with the following exception: Periodic analyses of well water for potential contaminants were not performed according to GLP standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.

A. MATERIALS:

1. Test material BAS 781 02 H (formulation containing 54.6% Dimethenamid-P and 6.2% Saflufenacil)

Description: Liquid

Lot No./Batch No. : 1632-78 (Batch No.)

Purity: 54.6% BAS 656 H and 6.2% BAS 800 H

Stability of compound under test conditions: The time 0 measured concentrations of BAS 781 02 H yielded recoveries of 102 to 108% of nominal test concentrations, while the 96-hour measured concentrations yielded recoveries of <LOQ to 55.2% of nominal test concentrations, indicating that the test material was not stable under test conditions. However, the measured test material (Dimethenamid-P) was stable in other freshwater studies at the same general test concentrations, and the decline of the test substance appeared to be correlated to the number of algal cells, suggesting potential absorption of the test substance to the algal cells.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored under ambient conditions.

Physicochemical properties of BAS 781 02 H (AI: Saflufenacil).

Parameter	Values	Comments
Water solubility at 20°C	**Not reported.**	
Vapor pressure	**Not reported.**	
UV absorption	**Not reported.**	
pKa	**Not reported.**	
Kow	**Not reported.**	

2. Test organism:

Name: Freshwater algae, *Pseudokirchneriella subcapitata*
EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: Not reported
Source: In-house cultures originally obtained from the University of Toronto Culture Collection
Age of inoculum: At least 2 weeks
Method of cultivation: Grown under test conditions (freshwater algal medium) in a temperature-controlled environmental chamber

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was conducted with a negative control and nominal test concentrations of 8.1, 27, 90, 300, and 1000 µg/L. At 96 hours, inhibitions in the treated cultures as compared to the negative control were 27, 96, 99, 99, and 100%, respectively.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	Continuous	
Culturing media and conditions:	Freshwater algal medium	

Parameter	Details	Remarks
		Criteria
(same as test or not)	same as test	*EPA recommends two week acclimation period.*
Health: (any mortality observed)	Pretest health was not reported.	*OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system Static/static renewal	Static	
Renewal rate for static renewal	N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Test vessels were placed on mechanical shakers in a temperature controlled environmental chamber.	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name		

Parameter	Details	Remarks *Criteria*
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	7.5-7.6 7.9-9.1 Yes $NaHCO_3$ N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes.	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Purified well water Adjusted to 7.4. N/A. Filter sterilized. Not reported. Not reported. See Reviewer's Comments. None Detected. Not reported.	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	A secondary stock solution was prepared using a primary stock solution to obtain a nominal concentration of 30 µg/L. This secondary stock solution was then serially diluted with freshwater algal medium to obtain the remaining lower test concentrations.	
Aeration or agitation	Agitation (100 rpm).	
Initial cells density	$1.0x10^4$ cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Parameter	Details	Remarks *Criteria*
Number of replicates Control: Solvent control: Treatments:	3 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations Nominal:	0 (negative control), 3.9, 6.5, 11, 18, and 30 µg form/L	Only concentrations of dimethenamid-p were analytically verified. Given the stability of saflufenacil and dimethenamid-p in freshwater studies and lack of analytical verification for the formulation, all endpoints are reported in terms of nominal concentration. *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples, and matrix and calibration samples collected at 0 and 96 hours were analyzed using HPLC with UV (240 nm) detection.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 23.4-25.7°C Continuous 4080-4560 lux Cool-white fluorescent lights	 *EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	 N/A N/A	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	-cell density -area under the growth curve (biomass) -growth rate -yield	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell counts were conducted daily on all replicate vessels of each test concentration and the controls using an electronic particle counter (Coulter Electronics, Inc.). Area under the curve was determined by an equation that took into account the daily cell density from Day 0 to 4. Growth rate was determined by an equation that evaluated the change in cell density from Day 0 to 4. Yield was determined by subtracting initial cell density from final cell density.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	Refer to Inhibitory Effects.	
Indicate whether there was an exponential growth in the control	Yes. After 96 hours, the mean cell density was 389.8 x 10^4cells/mL in the negative control.	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours of exposure, cell density averaged 389.8 x 10^4 cells/mL in the negative control, yielding inhibitions of 2.0, 9.1, 22, 65, and 96% as compared to the negative control at the nominal 3.9, 6.5, 11, 18, and 30 µg form/L treatment levels, respectively. The 96-hour NOAEC and EC_{50} values based on cell density were reported by the study authors to be 4.0 and 16 µg/L, respectively.

After 96 hours of exposure, area under the curve (biomass) averaged 916.2 x 10^5 cells/ mL*hour in the negative control, yielding inhibitions of 5.8, 9.8, 27, 72, and 96% as compared to the negative control. The 96-hour NOAEC and EC_{50} values based on biomass were reported by the study authors to be 4.0 and 15 µg/L, respectively.

Growth rate averaged 0.0621 cells/mL/hour in the negative control, yielding inhibitions of 0.33, 1.6, 4.2, 18, and 55% as compared to the negative control. The 96-hour NOAEC and EC_{50} values based on growth rate were reported by the study authors to be 11 and 29 µg/L, respectively.

Yield averaged 388.8 x 10^4 cells/mL in the negative control, yielding inhibitions of 2.0, 9.1, 22, 65, and 96% as compared to the negative control. The 96-hour NOAEC and EC_{50} values based on yield were reported by the study authors to be 4.0 and 17 µg/L, respectively.

There were no signs of flocculation or aggregation of cells and no adherence to the test vessels in the controls and treatment groups. Cell enlargement was observed in the highest treatment level.

Table 3: Effect of BAS 781 02 H (AI: Saflufenacil) on algal growth (*Pseudokirchneriella subcapitata*)

Initial Measured and (Nominal) Concentrations (µg/L)	Initial cell Density (x10^4 cells/mL)	Cell density (x10^4 cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					cell count	% inhibition
Negative control	1.0	5.6	29.1	155.7	389.8	NA
3.97 (3.9)	1.0	4.7	26.6	141	382.1	2.0
7.0 (6.5)	1.0	4.2	20.7	145.7	354.2	9.1
11.4 (11)	1.0	4.7	18.2	109.2	303.8	22
19.1 (18)	1.0	3.0	7.0	29.7	137.8	65
30.7 (30)	1.0	2.9	4.0	6.0	15.0	96

Table 4: Effect of BAS 781 02 H on algal growth (*Pseudokirchneriella subcapitata*)

Initial Measured and (Nominal) Concentrations (µg/L)	Initial Cell Density ($x10^4$ cells/mL)	Mean Growth Rate (cells/mL/hour)		Mean Area Under the Growth Curve (Biomass) ($x\ 10^5$ cells/mL*hour)	
		0-96 Hours	Percent Inhibition	0-96 hours	Percent Inhibition
Negative control	1.0	0.0621	N/A	916.2	N/A
3.97 (3.9)	1.0	0.0619	0.33	863.5	5.8
7.0 (6.5)	1.0	0.0611	1.6	826.3	9.8
11.4 (11)	1.0	0.0595	4.2	673.4	27
19.1 (18)	1.0	0.0512	18	252.2	72
30.7 (30)	1.0	0.0278	55	40.3	96

N/A- Not Applicable

Table 5: Statistical endpoint values (Expressed in terms of BAS 781 02 H)*.

Statistical Endpoint	Cell density	Biomass (Area under the Growth Curve)	Growth Rate	Yield
NOAEC or EC_{05} (µg/L)	4.0	4.0	11	4.0
EC_{50} (µg/L)	16	15	29	17
IC_{50} or EC_{50} (µg/L) (95% C.I.)	16 (15-17)	15 (14-16)	29 (28-30)	17 (15-17)
Other (EC_{90})	ND	ND	ND	ND
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A	N/A

*Study author-reported values.

B. REPORTED STATISTICS:

Statistical analysis was performed for the endpoints of cell density, biomass (area under the growth curve), growth rate, and yield using SAS Version 8.2. Non-linear regression or linear interpolation was used to calculate EC_{50} values and their corresponding confidence intervals when possible. The data were tested for normality using Shapiro-Wilks' Test, and for homogeneity of variance using Levene's Test. If the assumptions were not met, an

attempt was made to correct the data with log transformation. If the transformations were not effective, ANOVA and Dunnett's test were still used to make the comparisons. The results of the statistical analyses and an evaluation of the concentration-response pattern were used to determine the NOAEC values.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Replicate data for all endpoints were tested for normality and homogeneity. If the assumptions of ANOVA were met, the NOAEC value was determined using the parametric Dunnett's and William's Test. If the assumptions were not met, the NOAEC value was determined using the non-parametric Steels or Kruskal-Wallis Test. All NOAEC values were determined using Toxstat Statistical Software. ECx values (with 95% C.I.) and probit slopes were determined using probit analyses via Nuthatch Statistical Software. All toxicity values were determined using the nominal concentrations. Cell density and yield values were divided by 10,000, area under the growth curve values were divided by 100,000, and growth rate values were multiplied by 10,000 before entry into Toxstat.

BAS 781 02 H

Cell density

EC_{05}: 8.3 µg form/L 95% C.I.: 7.6 to 9.2 µg form/L
EC_{50}: 16 µg form/L 95% C.I.: 15 to 16 µg form/L
NOAEC: 3.9 µg form/L
Probit Slope: 6.05 ± 0.293

Biomass (Area Under the Growth Curve)

EC_{05}: 7.1 µg form/L 95% C.I.: 6.8 to 8.5 µg form/L
EC_{50}: 14 µg form/L 95% C.I.: 14 to 15 µg form/L
NOAEC: 3.9 µg form/L
Probit Slope: 5.40 ± 0.279

Growth Rate

EC_{05}: 13 µg form/L 95% C.I.: 11 to 15 µg form/L
EC_{50}: 28 µg form/L 95% C.I.: 28 to 30 µg form/L
NOAEC: 11 µg form/L
Probit Slope: 4.80 ± 0.331

Yield

EC_{05}: 8.4 µg form/L 95% C.I.: 7.6 to 9.3 µg form/L
EC_{50}: 16 µg form/L 95% C.I.: 15 to 16 µg form/L
NOAEC: 3.9 µg form/L
Probit Slope: 6.13 ± 0.303

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

There was significant degradation of dimethenamid-p in the formulation over the course of the study (BAS 781 02 H was reduced by *ca.* 40-50% across all treatment levels), indicating that dimethenamid-p was not stable under test conditions. However, the measured test material (dimethenamid-P) was stable in other freshwater studies at the same general test concentrations. The decrease in concentrations of dimethenamid-p in the treatment groups over the 96-hour exposure period was not accompanied by the decrease in growth inhibition. However, the greater decline of dimethenamid-p in the treatment groups with the greatest number of algal cells indicated that the decline of test substance in the test solutions correlated to the number of algal cells in the treatment groups. Therefore, the study authors suggest that the decline in dimethenamid-p concentrations was possibly due to the absorption of test substance to the algal cells. As a result, the nominal concentrations were used for all toxicity calculations.

Concentrations of Saflufenacil (BAS 800H) were not measured in this study. The analytical determination was conducted for the primary active ingredient, Dimethenamid-P, only. Analytically verified concentrations of dimethenamid-p were 102-108% of nominal on day 0. Although the stability of saflufenacil was not measured under test conditions, it is expected that it is stable given measured concentrations were 104-108% of nominal on day 0 and 96-105% after 96 hrs in the green algae study conducted with saflufenacil (IIA.8.4; MRID: 47127923; PMRA: 1547225). Considering dimethenamid-p was stable, saflufenacil is expected to be stable, and concentrations of the whole formulation were not measured, all biological endpoints are reported in terms of nominal concentrations.

The reviewer's results agreed with the study authors' results.

It should be noted that the NOAEC endpoint for growth rate (11 µg form/L) was based on visual determination because the 18% inhibition in growth rate at the 18 µg form/L treatment level was considered to be biologically significant.

The following conditions for the validity of the test were met:

1. The mean cell density in the control flasks increased by a factor greater than 16 within three days.
2. The coefficient of variation of average specific growth rate in the control replicates during the whole test period did not exceed 7%. It was 0.46%.
3. The mean percent coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, and 2-3) did not exceed 35%. It was 28%.

Results from the periodic screening analysis of the dilution water indicated the presence of the following components: calcium (38.7 mg/L), chloride (4.2 mg/L), fluoride (0.55 mg/L), magnesium (14.6 mg/L), potassium (6.97 mg/L), sodium (19.8 mg/L) and sulfate (6.0 mg/L).

All test solutions appeared clear and colorless.

The in-life portion of the definitive algal toxicity test was conducted between August 4 and 8, 2008.

F. CONCLUSIONS:

The study is scientifically sound and is classified as ACCEPTABLE to the U.S. EPA and as FULLY RELIABLE to PMRA and APVMA. The most sensitive endpoint was biomass, with NOAEC and EC_{50} values of 3.9 and 14 µg

form/L, respectively.

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

BAS 781 02 H
Cell density
EC_{05}: 8.3 µg form/L 95% C.I.: 7.6 to 9.2 µg form/L
EC_{50}: 16 µg form/L 95% C.I.: 15 to 16 µg form/L
NOAEC: 3.9 µg form/L
Probit Slope: 6.05 ± 0.293

Biomass (Area Under the Growth Curve)
EC_{05}: 7.1 µg form/L 95% C.I.: 6.8 to 8.5 µg form/L
EC_{50}: 14 µg form/L 95% C.I.: 14 to 15 µg form/L
NOAEC: 3.9 µg form/L
Probit Slope: 5.40 ± 0.279

Growth Rate
EC_{05}: 13 µg form/L 95% C.I.: 11 to 15 µg form/L
EC_{50}: 28 µg form/L 95% C.I.: 28 to 30 µg form/L
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Yield
EC_{05}: 8.4 µg form/L 95% C.I.: 7.6 to 9.3 µg form/L
EC_{50}: 16 µg form/L 95% C.I.: 15 to 16 µg form/L
NOAEC: 3.9 µg form/L
Probit Slope: 6.13 ± 0.303

Endpoint(s) Effected: Cell density, biomass (most sensitive), growth rate, and yield

III. REFERENCES:

1. Official Journal of the European Communities. 1992. No. L383. Method C.3: *Algal Inhibition Test.*
2. Organization for Economic Cooperation and Development. 2006. Guidelines for Testing of Chemicals, Guideline 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test.* Adopted 23 March 2006.
3. U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II.*
4. ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static96-Hour Toxicity tests with Microalgae.* American Society for Testing and Materials. Philadelphia, PA.
5. Schulz, H., and M. Meyer. 2007. *Determination of Dimethenamid-P and Its Metabolites M23 and M27 in Tap and Surface Water – Validation of the Method 519/0.* SGS Institut Fresenius GmbH Project Number IF-07/00871632. BASF DocID 2007/1054384.
6. SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8.2. Cary, North Carolina, SAS Institute, Inc.

7. Bruce, Robert D. and Donald J. Versteeg. 1992. A Statistical Procedure for Modeling Continuous Toxicity Data. *Environmental Toxicology and Chemistry*. 11: 1485-1494.
8. Norberg-King, T.J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach.* Version 2.0. U.S. EPA. National Effluent Toxicity Center. Duluth, Minnesota. Technical Report 03-93.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     1.206         4.356             6.876           4.356       1.206
OBSERVED     0             6                 6               6           0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   3.7645
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D = 1498.569

W =    0.976

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   45.21
Closest, conservative, Table H statistic = 1362.0   (alpha = 0.01)

Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   6,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.
```

```
NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =    5.86
Table Chi-square value =   15.09  (alpha = 0.01)
Table Chi-square value =   11.07  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   5
--------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c       Transform: NO TRANSFORMATION

                                ANOVA TABLE
--------------------------------------------------------------------------------

SOURCE             DF              SS                MS              F
--------------------------------------------------------------------------------
Between             5          352424.695        70484.939        564.417

Within (Error)     12            1498.569          124.881
--------------------------------------------------------------------------------
Total              17          353923.265
--------------------------------------------------------------------------------

  Critical F value =   3.11  (0.05,5,12)
  Since  F > Critical F  REJECT  Ho:All groups equal


BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c       Transform: NO TRANSFORMATION

      DUNNETTS TEST  -   TABLE 1 OF 2            Ho:Control<Treatment
--------------------------------------------------------------------------------

                                TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION           MEAN          ORIGINAL UNITS    T STAT  SIG
----- --------------------    -----------    ------------------  ------  ---
  1           Neg control      389.763           389.763
  2                   3.9      382.113           382.113          0.838
  3                   6.5      354.227           354.227          3.895  *
```

4	11.0	303.827	303.827	9.418 *
5	18.0	137.660	137.660	27.630 *
6	30.0	14.963	14.963	41.077 *

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	3.9	3	22.811	5.9	7.650
3	6.5	3	22.811	5.9	35.537
4	11.0	3	22.811	5.9	85.937
5	18.0	3	22.811	5.9	252.103
6	30.0	3	22.811	5.9	374.800

BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	389.763	389.763	389.763
2	3.9	3	382.113	382.113	382.113
3	6.5	3	354.227	354.227	354.227
4	11.0	3	303.827	303.827	303.827
5	18.0	3	137.660	137.660	137.660
6	30.0	3	14.963	14.963	14.963

BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
File: 0403c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	389.763				
3.9	382.113	0.838		1.78	k= 1, v=12
6.5	354.227	3.895	*	1.87	k= 2, v=12
11.0	303.827	9.418	*	1.90	k= 3, v=12
18.0	137.660	27.630	*	1.92	k= 4, v=12

```
              30.0      14.963      41.077       *        1.93        k= 5, v=12
-------------------------------------------------------------------------------
s =   11.175
Note: df used for table values are approximate when v > 20.

Estimates of EC%
-------------------------------------------------------------------------
Parameter   Estimate      95% Bounds          Std.Err.   Lower Bound
                        Lower      Upper                  /Estimate
EC5              8.3        7.6        9.2      0.020        0.90
EC10             9.6        8.8         10.     0.018        0.92
EC25             12.         11.        13.     0.013        0.94
EC50             16.         15.        16.    0.0092        0.96

         Slope =      6.05  Std.Err. =     0.293


Goodness of fit: p =       0.16  based on DF=       3.0        12.
-------------------------------------------------------------------------
ALGDENS.TXT : BAS 781 02 H & P. subcapitata 96-hour cell density ug/L
-------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
-------------------------------------------------------------------------
     Dose      #Reps.      Obs.       Pred.       Obs.      Pred.    %Change
                           Mean       Mean      -Pred.    %Control

     0.00       3.00       390.       376.       13.6       100.       0.00
     3.90       3.00       382.       376.       6.03       100.     0.0134
     6.50       3.00       354.       372.      -17.9       98.9       1.07
     11.0       3.00       304.       309.      -5.03       82.1       17.9
     18.0       3.00       138.       133.       4.49       35.4       64.6
     30.0       3.00       15.0       16.2      -1.22       4.30       95.7


BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
-------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     1.206         4.356             6.876           4.356       1.206
OBSERVED     0             7                 4               7           0

-------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   6.8246
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b       Transform: NO TRANSFORMATION
```

```
Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D = 17017.092

W =    0.949

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   23.84
Closest, conservative, Table H statistic = 1362.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   6,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =    4.07
Table Chi-square value =   15.09  (alpha = 0.01)
Table Chi-square value =   11.07  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   5
--------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).
```

BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1980373.017	396074.603	279.301
Within (Error)	12	17017.092	1418.091	
Total	17	1997390.109		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	916.227	916.227		
2	3.9	863.540	863.540	1.714	
3	6.5	826.260	826.260	2.926	*
4	11.0	673.380	673.380	7.898	*
5	18.0	252.173	252.173	21.597	*
6	30.0	40.320	40.320	28.487	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	3.9	3	76.868	8.4	52.687
3	6.5	3	76.868	8.4	89.967
4	11.0	3	76.868	8.4	242.847
5	18.0	3	76.868	8.4	664.053
6	30.0	3	76.868	8.4	875.907

BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	916.227	916.227	916.227
2	3.9	3	863.540	863.540	863.540
3	6.5	3	826.260	826.260	826.260
4	11.0	3	673.380	673.380	673.380
5	18.0	3	252.173	252.173	252.173
6	30.0	3	40.320	40.320	40.320

BAS 781 02 H & P. subcapitata 96-hour biomass ug/L
File: 0403b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	916.227				
3.9	863.540	1.714		1.78	k= 1, v=12
6.5	826.260	2.926	*	1.87	k= 2, v=12
11.0	673.380	7.898	*	1.90	k= 3, v=12
18.0	252.173	21.597	*	1.92	k= 4, v=12
30.0	40.320	28.487	*	1.93	k= 5, v=12

s = 37.658
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	7.1	6.3	8.0	0.025	0.89
EC10	8.3	7.5	9.2	0.021	0.90
EC25	11.	10.	12.	0.016	0.92
EC50	14.	14.	15.	0.011	0.95

Slope = 5.40 Std.Err. = 0.279

Goodness of fit: p = 0.23 based on DF= 3.0 12.

ALGBIO~1.TXT : BAS 781 02 H & P. subcapitata 96-hour biomass ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change

0.00	3.00	916.	883.	33.2	100.	0.00
3.90	3.00	864.	882.	-18.5	99.9	0.111
6.50	3.00	826.	855.	-29.1	96.9	3.13
11.0	3.00	673.	649.	24.1	73.5	26.5
18.0	3.00	252.	265.	-12.5	30.0	70.0
30.0	3.00	40.3	37.5	2.78	4.25	95.7

BAS 781 02 H & P. subcapitata 96-hour growth rate ug/L
File: 0403g Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	6	5	7	0

Calculated Chi-Square goodness of fit test statistic = 5.1491
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

BAS 781 02 H & P. subcapitata 96-hour growth rate ug/L
File: 0403g Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 2653.333

W = 0.823

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

BAS 781 02 H & P. subcapitata 96-hour growth rate ug/L
File: 0403g Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 3259.00
Closest, conservative, Table H statistic = 1362.0 (alpha = 0.01)

```
Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   6,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data FAIL homogeneity test. Try another transformation.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


BAS 781 02 H & P. subcapitata 96-hour growth rate ug/L
File: 0403g       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =   24.59
Table Chi-square value =   15.09  (alpha = 0.01)
Table Chi-square value =   11.07  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   5
--------------------------------------------------------------------------------

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).




BAS 781 02 H & P. subcapitata 96-hour growth rate ug/L
File: 0403g       Transform: NO TRANSFORMATION

             KRUSKAL-WALLIS ANOVA BY RANKS  -  TABLE 1 OF 2
--------------------------------------------------------------------------------
                              TRANSFORMED   MEAN CALCULATED IN      RANK
GROUP    IDENTIFICATION         MEAN         ORIGINAL UNITS         SUM
----- --------------------    ----------   ------------------   ----------
  1           Neg control     621.333          621.333          49.000
  2                   3.9     619.000          619.000          44.000
  3                   6.5     611.667          611.667          33.000
  4                  11.0     595.667          595.667          24.000
  5                  18.0     512.333          512.333          15.000
  6                  30.0     278.333          278.333           6.000
--------------------------------------------------------------------------------

  Calculated H Value =  16.268         Critical H Value Table =  11.070
  Since  Calc H > Crit H  REJECT Ho:All groups are equal.
```

BAS 781 02 H & P. subcapitata 96-hour growth rate ug/L
File: 0403g Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
6	30.0	278.333	278.333	\
5	18.0	512.333	512.333	. \
4	11.0	595.667	595.667	. . \
3	6.5	611.667	611.667	. . . \
2	3.9	619.000	619.000	. . . . \
1	Neg control	621.333	621.333	* . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 4.357

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	13.	11.	15.	0.025	0.89
EC10	15.	14.	17.	0.020	0.91
EC25	20.	19.	22.	0.012	0.94
EC50	28.	27.	29.	0.0066	0.97

Slope = 4.80 Std.Err. = 0.331

Goodness of fit: p = 0.90 based on DF= 3.0 12.

ALGGROW.TXT : BAS 781 02 H & P. subcapitata 96-hour growth rate ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	621.	616.	5.04	100.	0.00
3.90	3.00	619.	616.	2.72	100.	0.00179
6.50	3.00	612.	616.	-3.96	99.9	0.108
11.0	3.00	596.	601.	-5.64	97.6	2.43
18.0	3.00	512.	510.	2.24	82.8	17.2
30.0	3.00	278.	279.	-0.395	45.2	54.8

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	6	6	6	0

Calculated Chi-Square goodness of fit test statistic = 3.7645
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1498.569

W = 0.976

Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858

Data PASS normality test at P=0.01 level. Continue analysis.

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 45.21
Closest, conservative, Table H statistic = 1362.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 2
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 5.86
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	352424.695	70484.939	564.417
Within (Error)	12	1498.569	124.881	
Total	17	353923.265		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	388.763	388.763		
2	3.9	381.113	381.113	0.838	
3	6.5	353.227	353.227	3.895	*
4	11.0	302.827	302.827	9.418	*
5	18.0	136.660	136.660	27.630	*
6	30.0	13.963	13.963	41.077	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

BAS 781 02 H & P. subcapitata 96-hour yield ug/L

File: 0403y Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	3.9	3	22.811	5.9	7.650
3	6.5	3	22.811	5.9	35.537
4	11.0	3	22.811	5.9	85.937
5	18.0	3	22.811	5.9	252.103
6	30.0	3	22.811	5.9	374.800

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	388.763	388.763	388.763
2	3.9	3	381.113	381.113	381.113
3	6.5	3	353.227	353.227	353.227
4	11.0	3	302.827	302.827	302.827
5	18.0	3	136.660	136.660	136.660
6	30.0	3	13.963	13.963	13.963

BAS 781 02 H & P. subcapitata 96-hour yield ug/L
File: 0403y Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	388.763				
3.9	381.113	0.838		1.78	k= 1, v=12
6.5	353.227	3.895	*	1.87	k= 2, v=12
11.0	302.827	9.418	*	1.90	k= 3, v=12
18.0	136.660	27.630	*	1.92	k= 4, v=12
30.0	13.963	41.077	*	1.93	k= 5, v=12

s = 11.175
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds	Std.Err.	Lower Bound

		Lower	Upper		/Estimate
EC5	8.4	7.6	9.3	0.021	0.90
EC10	9.6	8.8	11.	0.018	0.92
EC25	12.	11.	13.	0.013	0.94
EC50	16.	15.	16.	0.0093	0.96

Slope = 6.13 Std.Err. = 0.303

Goodness of fit: p = 0.15 based on DF= 3.0 12.

ALGYLD.TXT : BAS 781 02 H & P. subcapitata 96-hour yield ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	389.	375.	14.0	100.	0.00
3.90	3.00	381.	375.	6.35	100.	0.0111
6.50	3.00	353.	371.	-17.9	99.0	0.984
11.0	3.00	303.	309.	-6.02	82.4	17.6
18.0	3.00	137.	132.	4.91	35.2	64.8
30.0	3.00	14.0	15.3	-1.31	4.07	95.9